A glucose biosensor based on modified-enzyme incorporated within electropolymerised poly(3,4-ethylenedioxythiophene) (PEDT) films

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A glucose biosensor based on modified-enzyme incorporated within electropolymerised poly(3,4-ethylenedioxythiophene) (PEDT) films

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Abstract

We have constructed and characterised a glucose sensor using glucose oxidase (GOD) covalently attached to carboxylic acid polyethyleneglycol (PEG), called (PEG–GOD). This modified enzyme was entrapped afterwards within poly(3,4-ethylenedioxythiophene) (PEDT) films electrogenerated on glassy carbon (GC) electrodes. The composite (PEG–GOD/PEDT) film is more porous than the film without enzyme (PEDT+PEG). Data from electrochemical quartz microbalance (ECQM) and pH-stat experiments indicate a good relative activity of the modified enzyme, *ca.* 12–15%. Amperometric measurements, using ferrocenemethanol as the redox mediator, confirms that the modified enzyme is catalytically active. The effect of film thickness was also investigated. The sensitivities were quite similar for modified-GOD electrodes (*ca.* 3 mA cm^{-2} M^{-1}) and unmodified-GOD electrodes (*ca.* 2.7 mA cm^{-2} M^{-1}) but a better stability was obtained with modified PEG–GOD electrodes.

Keywords:

Biosensors; Covalent immobilisation; Conducting polymers; Glucose; Electrochemical quartz microbalance

I. Introduction

Amperometric biosensors based on immobilisation of enzymes in an electronically conducting polymer (ECP) matrix are of great interest [1], [2], [3], [4], [5] and [6]. ECPs can be grown selectively on a conductive surface allowing miniaturisation of the biosensor. The properties of the polymerised film can be easily controlled (porosity, thickness) by electrochemical procedures. Moreover, ECPs can be chemically modified to improve the affinity towards enzymes, eventually to form covalent bonds.

Two conventional procedures exist for immobilisation of enzymes into ECP-modified electrodes. The first consists of the entrapment of the bulky enzyme within the polymer matrix during its electropolymerisation [7], [8] and [9]. The second consists of two steps: ECP is first grown on the electrode surface and then the enzyme is allowed to react chemically with the polymer surface by covalent bonds or by bioaffinity interactions [9] and [10]. The physical entrapment is the simplest way but the catalytic activity of the enzymes is drastically reduced down to a few percent of the value observed in the bulk [11]. The enzyme attachment on a pre-

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polymerised film is a more complicated technique, which limits the enzyme quantity to a few monolayers at the film | solution interface and the activity is also reduced. Nevertheless, the stability is increased [12] and [13].
In general, the direct electron transfer between the electrode and the active site of the enzyme is difficult, and a redox mediator (cosubstrate) is needed in the catalytic cycle. Glucose oxidase (GOD), the most common oxidase enzyme, needs molecular oxygen as a cosubstrate for its reoxidation after reaction with the glucose substrate, in the following manner:

$$\text{Glucose} + \text{GOD(ox)} \rightarrow \text{Gluconolactone} + \text{GOD(red)}$$

$$\text{GOD(red)} + O_2 \rightarrow \text{GOD(ox)} + H_2O_2$$

Oxygen is consumed and hydrogen peroxide is produced. Because oxygen cannot be available in all media with the same concentration and because conducting polymers tend to deteriorate in the presence of H_2O_2, it would be advantageous to replace oxygen by another mediator (Med):

$$\text{GOD(red)} + \text{Med(ox)} \rightarrow \text{GOD(ox)} + \text{Med(red)}$$

$$\text{Med(red)} \rightarrow \text{Med(ox)} + e^-$$

Recently, we have developed a composite film, [poly(3,4-ethylenedioxythiophene) (PEDT)+polyethyleneglycol (PEG)] (PEDT+PEG), by electrochemical oxidation of EDT in the presence of PEG in phosphate buffer. It was shown that the composite is excellent for the entrapment and delivery of very large biomolecules such as oligonucleotides (ODN), in view of in vivo applications [14]. Moreover, the polymer composite seems to be biocompatible. These excellent results motivated us to use the composite (PEDT+PEG) to construct an enzyme-modified electrode.
The enzyme GOD has already been immobilised in conducting polymers, in particular polypyrrole [15], [16], [17], [18] and [19], but also polythiophene [20]. GOD has also been adsorbed on porous membranes containing polypyrrole, results indicating a direct electron transfer between GOD and polypyrrole [21], [22] and [23]. However, controversial data exist about this direct transfer [18], [24] and [25]. More recently, glucose was detected in a deaerated medium on a platinum electrode coated with polypyrrole [26], but at high potential, and the direct transfer was not obvious.
Keeping in mind that the more the polymer is conducting, the more the direct electron transfer can occur, and considering that PEDT has been described in the literature as one of the most conductive ECPs [27], we attempted then to immobilise GOD in a PEDT matrix, with the hope that a direct electron transfer could be achieved.
In this work, we describe the elaboration and characterisation of a glucose sensor using GOD. The enzyme was first covalently bonded to a polyethyleneglycol bearing a carboxylic acid group. The modified enzyme (PEG–GOD) was then entrapped in the PEDT matrix during electropolymerisation of EDT in phosphate buffer containing PEG–GOD. PEG plays the role of surfactant for the EDT monomer (it was indeed observed that PEG enhances EDT solubility).
The GOD immobilised under these conditions presents a better catalytic activity than that habitually observed in the literature [9], estimated by two methods: pH-stat for the enzyme activity [28] and ECQM measurements for the enzyme loading. Because amperometric tests without mediator during glucose monitoring are not satisfactory to demonstrate a direct electron transfer, we have used a ferrocene derivative (ferrocenemethanol, FcOH) as mediator to complete the study of the sensor.

II. Experimental

II.1. Chemicals

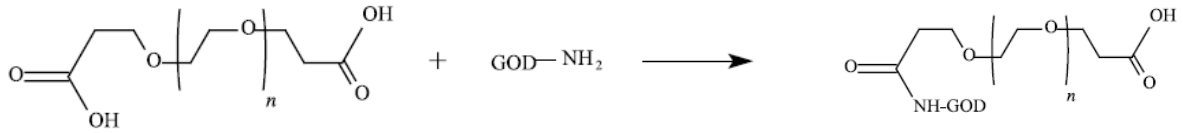
Glucose oxidase from *Aspergillus niger* (type VII-S, EC 1.1.3.4), D(+)glucose, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS)

were purchased from Sigma. Reagent grade KH_2PO_4, K_2HPO_4 and KCl were purchased from Prolabo. Dicarboxylic acid polyethylene glycol (PEG MM 600) was supplied by Fluka. 3,4-Ethylenedioxythiophene (EDT) was from Bayer. Ferrocenemethanol (FcOH) was from Aldrich. All reagents were used without purification; all solutions were prepared with bi-distilled water. Phosphate buffer (5×10^{-2} M, pH 6) contains 5×10^{-2} M KCl in all experiments. Glucose stock solution (0.25 M) was allowed to mutarotate for at least 24 h and stored at 4 °C prior to use.

II.2. Methods

II.2.1. Modification of GOD

Glucose oxidase bears amine groups on the proteinic envelope. The modification of GOD was achieved by forming peptide bonds between the amine groups of GOD and the carboxylic acid groups of PEG (scheme shown below). This kind of coupling has often been described in the literature [26], [29], [30] and [31].



A 5 ml phosphate buffer (pH 6.0) solution containing GOD (1000 IU ml^{-1}), with an excess of PEG MM 600 (10^{-4} M), EDC (1.5×10^{-4} M) and NHS (3×10^{-4} M), was prepared and stirred for 2 h.

Experiments were performed by varying the PEG/GOD ratio between 1:1 and 50:1. The 1:1 ratio gave no visible difference with the non-modified enzyme, whereas the 50:1 ratio led to a very deactivated enzyme. This could be explained by a steric hindrance of the PEG chains cross-linked on the enzyme, which impedes diffusion of glucose towards the FAD active centre [26] and [32]. A ratio between 3:1 and 4:1 gave the best result.

After the reaction, the solution was dialysed against 0.05 M phosphate buffer for 48 h to eliminate reagents. Dialysis was performed using a 6000 MM cut-off dialysis membrane. The remaining solution, containing the PEG-modified enzyme (PEG–GOD), was stored in a freezer when not in use.

II.2.2. Film elaboration

All the electrochemical studies were carried out using a single-compartment cell with a glassy carbon working electrode, a platinum gauze counter electrode and an AgCl covered Ag wire as the reference electrode (Ag | AgCl).

Glassy carbon electrodes (area 0.07 cm^2) were polished prior to use on a polishing cloth (Struers) sequentially with diamond paste of decreasing particle size (3 and 1 μm), rinsed with ethanol, ultrasonicated for 2 min in ethanol, and then air dried.

Two different experiments were used to construct the enzyme electrodes. The first case consists in electropolymerisation of EDT in phosphate buffer containing PEG and GOD. The second consists, as a first step, in the modification of GOD with PEG (described above, leading to the modified enzyme PEG–GOD) and as a second step, electropolymerisation of EDT in phosphate buffer containing PEG–GOD.

PEDT electrodes containing PEG and GOD (denoted as GOD/PEDT+PEG in the following) were obtained by electrooxidation at a constant potential of 1.1 V versus Ag | AgCl of 10^{-2} M EDT in 5 ml phosphate buffer (pH 6)+10^{-4} M PEG+1000 IU ml^{-1} GOD. Electrodes containing modified PEG–GOD (denoted as PEG–GOD/PEDT) were obtained by electrooxidation of 10^{-2} M EDT at the same potential, in 5 ml phosphate buffer+1000 IU ml^{-1} PEG–GOD. Other concentrations of GOD were used, up to 50 000 IU ml^{-1}. It appeared that in the last case, what we call the specific activity of the enzyme decreased. The electrodes were then constructed using 1000 IU ml^{-1} GOD.

II.2.3. Amperometric determination

The performances of enzyme electrodes for the detection of glucose were investigated. The amperometric responses of the enzyme electrodes to glucose injections in a 10 ml phosphate

buffer (pH 6.0) solution containing 3×10^{-4} M FcOH were examined by measuring the anodic current at a potential of 0.3 V (*vs.* Ag|AgCl) at room temperature under argon. All the solutions used in the amperometric studies were deaerated by bubbling argon for 15 min prior to use. Other experiments were conducted under aerated conditions, in order to evaluate the oxygen sensitivity of our electrode. The background current was allowed to settle to a constant baseline, and successive additions of 0.04 ml stock glucose solution (0.25 M) were injected, with gentle stirring for 5 s after each addition. The current as a function of time was monitored continuously until a steady state value was reached. The spike occurring after each addition of glucose is due to the stirring of the solution, which can be assumed as a transient state where the concentrations of glucose and mediator at the electrode vicinity would be higher than at the equilibrium state. These spikes are usually observed when ferrocenemethanol is used as mediator. When dioxygen is used as mediator, spikes are lower.

II.2.4. Electrochemical quartz microbalance (ECQM) measurements

ECQM experiments were conducted on a platinum sputtered AT-cut quartz crystal (0.2 cm^2, nominal frequency 9 MHz) with an EG&G 273A potentiostat and an EG&G-SEIKO QCA917 frequency analyser. Frequency variations are related to mass change following the Sauerbrey equation.

$$\Delta f = -\left(\frac{2}{\rho v}\right) f_0^2 \frac{\Delta m}{A}$$

The sensitivity $\Delta m/\Delta f = -1.1\times10^{-9}$ g Hz^{-1} is an experimental value determined by the manufacturer.

II.2.5. pH-stat

The pH-stat method was used to determine the apparent enzyme concentration of the modified electrodes. As mentioned in Section I, the oxidation of glucose produces gluconolactone and then gluconic acid, therefore this enzymatic reaction results in a change in pH. The enzyme electrode and pH electrode are immersed in a solution containing the substrate, in this case glucose. Glucose reacts with the immobilised GOD and is converted into gluconic acid, with the production of H^+. An alkaline solution (10^{-4} M KOH, from a 1 M KOH volumetric solution, Titrinorm, Prolabo) is added sequentially in the solution in order to maintain pH at a constant value of 7.4. Thus, the exact quantity of gluconic acid is monitored versus time, and then the enzyme activity is deduced. The enzyme activity EA (μmol min^{-1}) is obtained as follows:

$$EA = 10^3 c_t s \qquad (1)$$

where c_t is the alkali concentration, *i.e.* 10^{-4} mol l^{-1} and s is the averaged slope of the curve $V_{alkali} = f(t)$/ml min^{-1}.

III. Results and discussion

III.1. Film characterisation

III.1.1. FTIR spectroscopy

Spectra were recorded on a computer-controlled Nicolet 860 FTIR spectrometer. Films, electrosynthesised on GC plates, were analysed by the reflection mode. Figure 1 shows spectra of PEDT (a) and (PEG–GOD/PEDT) (b) films. There are no significant differences between spectra a and b. The main bands [33] are situated at 916 and 830 (C—S stretch), 1046 (—C—OROC— stretch), 1182 cm^{-1} (P=O stretch of phosphate anions). The bands at 1323, 1393 and 1514 cm^{-1} can be attributed to C=C in-plane ring vibrations. The band at 1668 cm^{-1} is due to the dopant anions HPO_4^{2-} and $H_2PO_4^-$. It can be concluded from the above IR data that the polymer matrix is effectively PEDT even when the modified enzyme has been incorporated.

III.1.2. Scanning electron microscopy (SEM)

The films thickness was determined with SEM, by stripping a piece of polymer from the electrode to reveal a cross-section of the film. The incidence angle used with the microscope was 80°. Figure 2a and b shows a (PEDT+PEG) film and a (PEG–GOD/PEDT) film obtained on a GC electrode by the potentiostatic method for 10s. The thicknesses of these films are equivalent at *ca.* 40 nm (Figure 3), which means that the faradaic yield of electropolymerisation is approximately the same in both cases.

III.1.3. pH-stat

The enzymatic activity was evaluated using the pH-stat method for the two kinds of films: with unmodified (GOD/PEDT+PEG) and modified (PEG–GOD/PEDT) films. The surface concentration of the active enzyme Γ^{active} can be estimated by the following relation:

$$\Gamma_{GOD}^{active} = \frac{EA \; x \; m_{eu}}{M_{GOD} \; x \; A} \qquad (2)$$

where EA is the enzyme activity of relation (1) /µmol min^{-1}, m_{eu} is the mass of one enzyme unit, *i.e.* 2.15×10^{-5} g min µmol^{-1} at pH 7.4 and 22 °C, M_{GOD} is the molar mass of the enzyme, *i.e.* 186 000 g mol^{-1}, and A is the electrode surface, *i.e.* 2.5 cm^2.
The results give:
Γ_{GOD}^{active} =0.9±0.1×10^{-12} mol cm^{-2} for (GOD/PEDT+PEG) and
Γ_{GOD}^{active} =1.1±0.1×10^{-12} mol cm^{-2} for (PEG–GOD/PEDT) electrodes. The enzyme activity is slightly higher for the modified enzyme.

III.1.4. Electrochemical quartz microbalance (ECQM)

To determine the quantity of enzyme in each film, EQCM experiments were performed. The masses of each kind of film were compared to evaluate the surface concentrations of unmodified GOD (Γ_{GOD}^{app}) and modified PEG–GOD ($\Gamma_{PEG\text{-}GOD}^{app}$) embedded during electropolymerisation.
During these experiments, the quartz admittance was monitored. Without a film, the admittance equals 4.58×10^{-3} Ω^{-1} (in phosphate buffer+10^{-2} M EDT+GOD+PEG). This admittance never drops below 95% of the initial value during experiments. Thus, the rigidity constraint can be assumed.
At a constant electropolymerisation charge, and assuming that GOD, PEG and PEG–GOD do not impede the polymerisation (see Section III.1.2), Γ_{GOD}^{app} in the case of unmodified enzyme, can be found by the relation given below:

$$\Gamma_{GOD}^{app} = \frac{m - m_{PEDT+PEG}}{M_{GOD} \; x \; A} \qquad (3)$$

where m is the mass of the film/µg, $m_{PEDT+PEG}$ is the mass of a (PEDT+PEG) film obtained with the same charge/µg, M_{GOD} is the molar mass of GOD, *i.e.* 186 000 g mol^{-1}, and A is the electrode surface, *i.e.* 0.2 cm^2.
In the case of the modified enzyme, Γ_{GOD}^{app} can be found by the same relation, assuming that the same quantity of PEG is incorporated in both cases. Curves $m=f(t)$ are presented in Figure 4. The results give Γ_{GOD}^{app} =7.1±0.2×10^{-12} mol cm^{-2} for (GOD/PEDT+PEG) and $\Gamma_{PEG\text{-}GOD}^{app}$ =7.1±0.2×10^{-12} mol cm^{-2} for (PEG–GOD/PEDT) electrodes. It can be noted that equivalent amounts of enzyme are found in both cases. By comparing the values of concentrations of GOD present in the polymer matrix with that of the active GOD concentrations obtained from pH-stat experiments, the yield y of active enzyme in the film (also called the specific activity of the enzyme) can be deduced:

$$y = \frac{\Gamma_{GOD}^{active}}{\Gamma_{GOD}^{app}} \; x \; 100 \qquad (4)$$

The results give y=12±2% for (GOD/PEDT+PEG) and y=15±2% for (PEG–GOD/PEDT) electrodes. These values are not very precise, so it is not possible to discuss the difference between (GOD/PEDT+PEG) and (PEG–GOD/PEDT) electrodes. Nevertheless, these activities are higher than those commonly obtained in the literature for this kind of enzyme entrapment [9].

III.1.5. Amperometry

The performances of the enzyme electrodes were investigated by chronoamperometry, under the conditions described in Section II.2.3. Figure 5 shows a typical amperometric response as a function of glucose concentration.

To evaluate the reproducibility, ten electrodes were prepared on different days using the same procedure. The variation is at most 8% across the entire concentration range, which shows a relatively good reproducibility.

It should be noted that a (PEDT+PEG) electrode without GOD did not show any response with glucose addition. Thus, the signals observed are effectively due to the enzyme incorporated in the polymer matrix.

The calibration curve for the amperometric response of glucose at the (PEG–GOD/PEDT) electrode is established in Figure 6a, which is linear up to 22 mM glucose. The sensitivities of the biosensors (determined by the slope of the calibration curve) attain a value of 2.7 mA cm^{-2} M^{-1} for the (PEG–GOD/PEDT) electrode and 3 mA cm^{-2} M^{-1} for (GOD/PEDT+PEG) (result not shown). These values are close to those commonly obtained in the literature for GOD entrapped in conducting polymers [34], often comprised between 2 and 5 mA cm^{-2} M^{-1}.

To evaluate the oxygen sensitivity of the (PEG–GOD/PEDT) electrode, the amperometric response was determined under the same conditions as previously, but in an aerated medium. The data presented in Figure 6b, show that the sensitivity is very similar to that obtained under deaerated conditions, *i.e.* 2.6 mA cm^{-2} M^{-1} (only the first points are shown). Nevertheless, the background current is larger when oxygen is present. The increase of this background current could be due to the reoxidation of hydrogen peroxide which comes from the GOD/oxygen side-reaction, even if this reoxidation is low on a GC electrode. When amperometry is performed at 0.3 V versus Ag | AgCl on a GC-modified electrode in an aerated solution without FcOH, a very low response current is observed, corresponding to less than 5% of the current obtained with FcOH.

Bartlett and coworkers [35] and [36] have established a relation between the current observed during chronoamperometric experiments and several kinetic parameters as well as the bulk glucose concentration:

$$I_{obs} = nFA\alpha\left(\frac{k_{cat}K_s s_\infty k K_A a_\infty \Gamma_{GOD}^{active}}{k_{cat}K_s s_\infty + k K_A a_\infty \left(K_s s_\infty + K_M\right)}\right) \qquad (5)$$

where I_{obs} is the measured current/A, n is the number of electrons exchanged per glucose molecule, *i.e.* two electrons, F is the Faraday constant, *i.e.* 96 487 C mol^{-1}, A is the electrode surface, here 0.07 cm^2, Γ_{GOD}^{active} is the surface coverage of the active enzyme/mol cm^{-2}, k is the rate constant for the reaction between enzyme and mediator, a_∞ is the bulk concentration of the mediator/mol dm^{-3}, K_S and K_A are the partition coefficients for glucose and mediator into the film, respectively, K_M and k_{cat} are the kinetic parameters for the reaction between enzyme and substrate, respectively, in mol dm^{-3} and s^{-1}, 1/α is the fraction of reduced mediator which reaches the electrode surface and s_∞ is the bulk concentration of glucose/mol dm^{-3}.

The calibration data of (PEG–GOD/PEDT) electrodes were fitted to Equation (5) written in the form of a hyperbolic function:

$$I_{obs} = nFA\alpha\left(\frac{P_1 s_\infty}{P_2 + s_\infty}\right)$$

An average value was found:

$$\frac{P_2}{P_1} = \frac{1}{nFA\alpha}\frac{K_M}{k_{cat}K_s\Gamma_{GOD}^{active}} = 6 \pm \ x\ 10^{-3} \qquad (6)$$

The kinetic parameters for the reaction between enzyme and substrate, K_M=33×10⁻³ mol l⁻¹, k_{cat}=800 s⁻¹ and αK_s~1, were taken from Reference [37]. Relation (6) gives Γ_{GOD}^{active} =5±1×10⁻¹³ mol cm⁻². This value is of the same order as that obtained above. Nevertheless, the difference could be explained by αK_s<1, *i.e.* a little loss of reduced mediator into the bulk, and/or a low diffusion of glucose into the film. The α value could be estimated using a rotating ring-disc electrode for example. A method has been proposed to evaluate it in polymerised phenols [32]. The aim of this paper is not to determine these parameters, but we paid particular attention to the film thickness.

III.1.6. Effect of film thickness

α and K_s are the two parameters which are most likely to be influenced by the film thickness. Moreover, it has often been shown that the active enzyme in a polymer film is localised near the polymer | solution interface [38] and [39]. Therefore, the critical parameter, as said above, should be the diffusion of the reduced mediator (characterised by α) or that of glucose (characterised by K_s) through the film. The effect of thickness has already been studied with polypyrrole. It was shown that the best amperometric activities were obtained for 0–1 μm thick films [40].

In our case, we have studied sensors with film thicknesses varying between 20 and 1000 nm. The sensitivities are reported in Figure 7 versus thickness. The sensitivity has a maximum value for thicknesses in the range of 20–10 nm and decreases with thicker films. Once again, according to Reference [41], this could be due to a low diffusion of the mediator or the substrate through the film. Nevertheless, when we tried to measure the diffusion coefficient of the mediator through the film (with respect to the thickness), we observed a very surprising result: a fraction of the reduced mediator is reoxidised directly on the PEDT film (the apparent diffusion coefficient increases with film thickness). Therefore, this seems to indicate that the limiting parameter is not the diffusion of the mediator.

III.1.7. Stability

The enzyme electrodes were also investigated for their operational stability with two kinds of electrodes containing unmodified and modified enzyme. Each electrode was used in ten experiments over 2 weeks (each point is an average of results from three different electrodes). In the case of the unmodified enzyme, the sensitivity decreases rapidly and is more than 50% lower at the end (Figure 8a). With the modified-enzyme electrode, the loss is only *ca.* 20% (Figure 8b).

IV. Conclusion

An amperometric glucose sensor was constructed by incorporation of modified-glucose oxidase within a conducting polymer matrix, poly(3,4-ethylenedioxythiophene) (PEDT). The enzyme (GOD) was modified by polyethyleneglycol (PEG) chains to create a hydrophilic environment for GOD. This was achieved by amide bonds formed between amine groups of GOD and carboxylic acid groups of dicarboxylic acid polyethyleneglycol molecules. The modified-enzyme was entrapped during electrochemical oxidation of EDT in phosphate buffer. Data from pH-stat experiments, ECQM measurements and amperometry indicate that the resulting glucose biosensors using ferrocene as mediator possess a good sensitivity (2.7 mA M⁻¹ cm⁻²) up to 22

mM and excellent reproducibility for film formation. The stability is better for modified-enzyme electrodes compared to that of electrodes with unmodified enzyme incorporated in a composite (PEDT+PEG).

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Figures

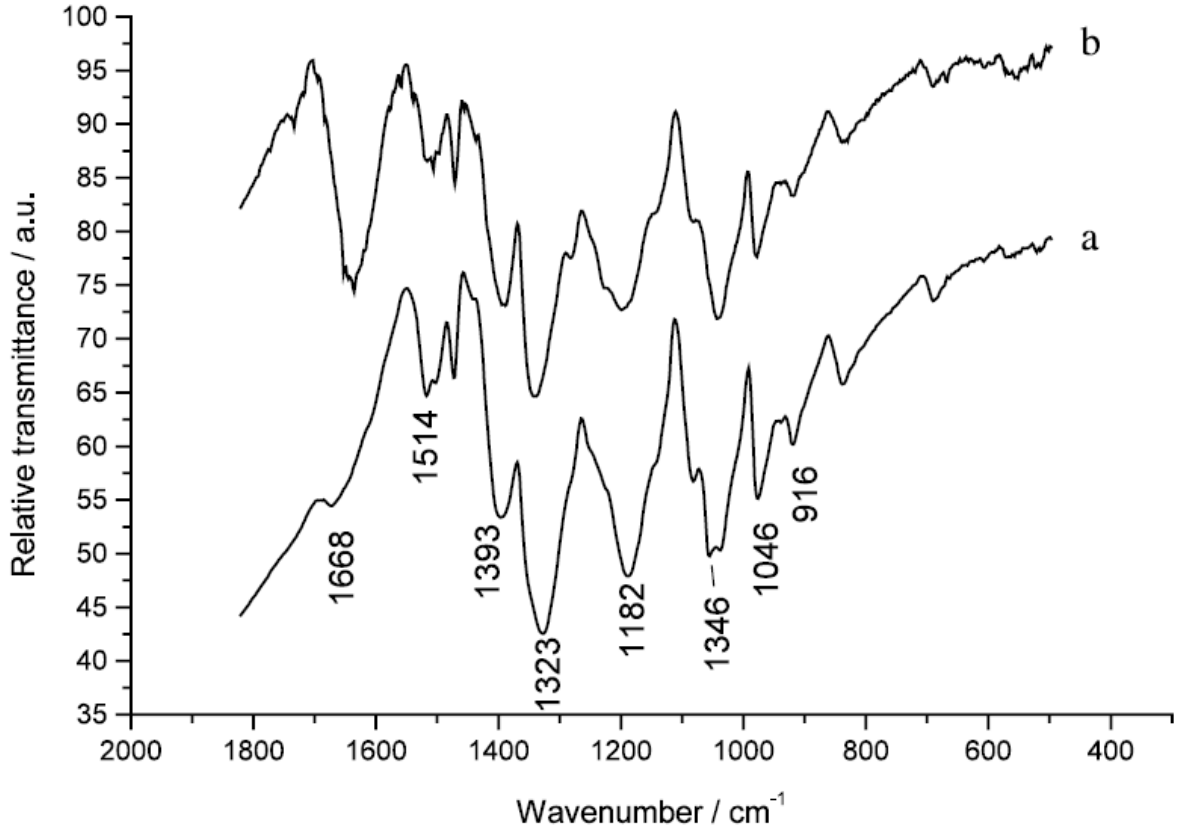
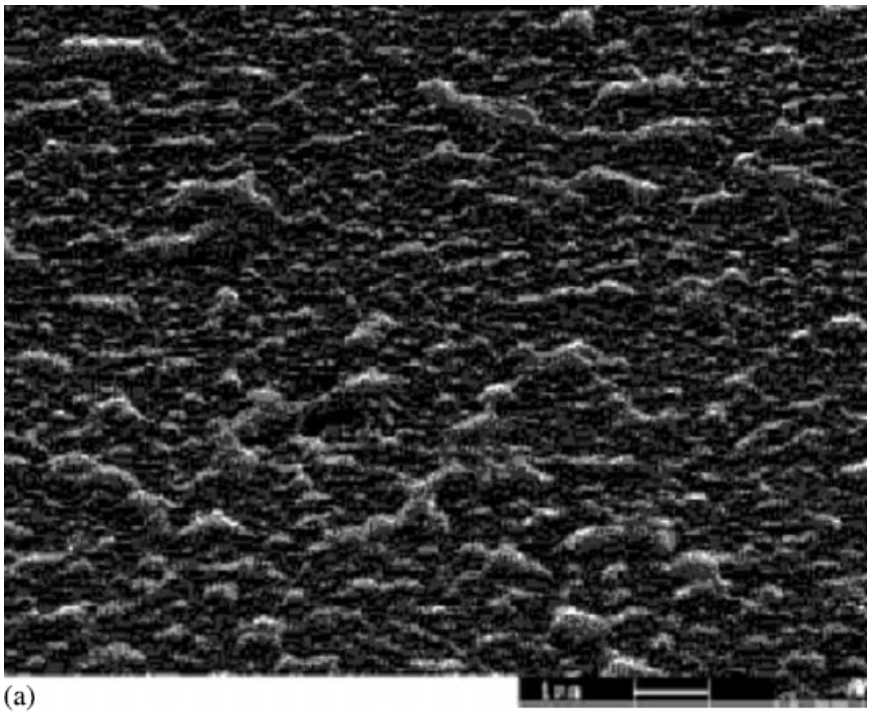
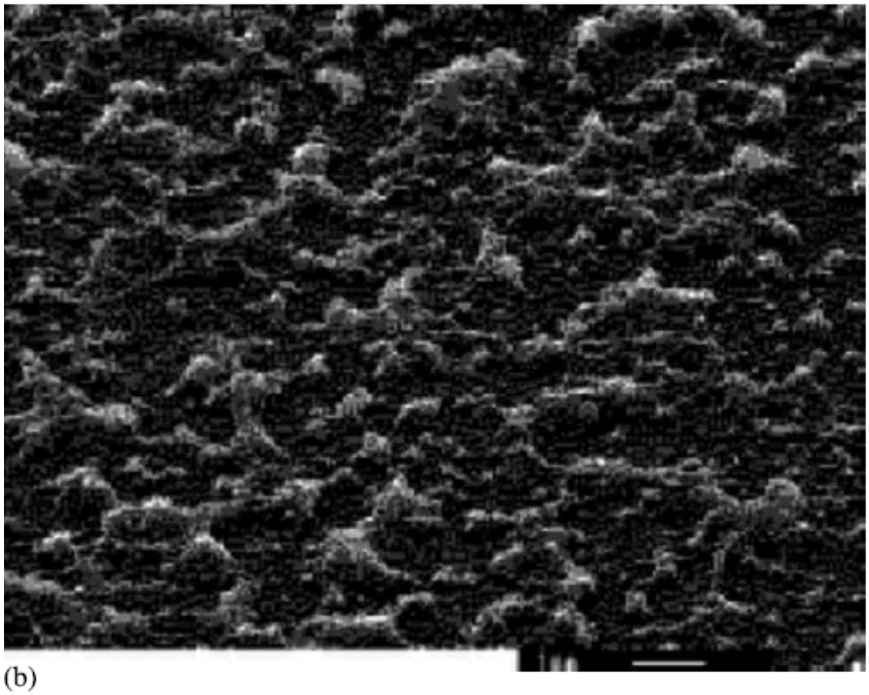


Figure 1: External reflection FTIR spectra of (a) PEDT alone and (b) PEDT film containing incorporated modified enzyme (PEG–GOD/PEDT).



(a)



(b)

Figure 2: SEM pictures of films electrodeposited on a GC electrode at constant potential +1.1 V for 10 s: (a) (PEDT+PEG) and (b) (PEG–GOD/PEDT).

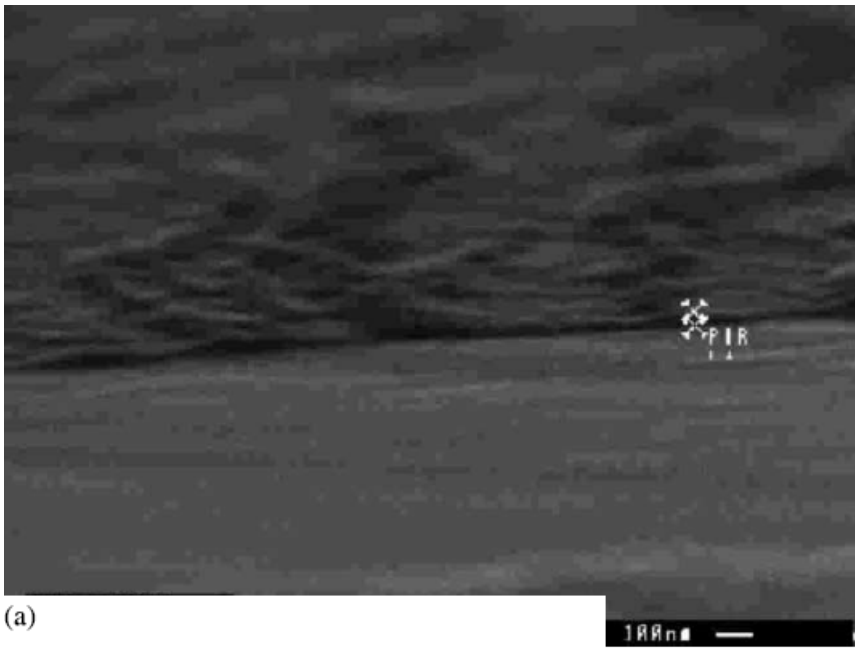


Figure 3: SEM pictures of the same films (a) and (b) in Figure 2. An 80° angle was used to measure the film thickness after a scratch was made on the film

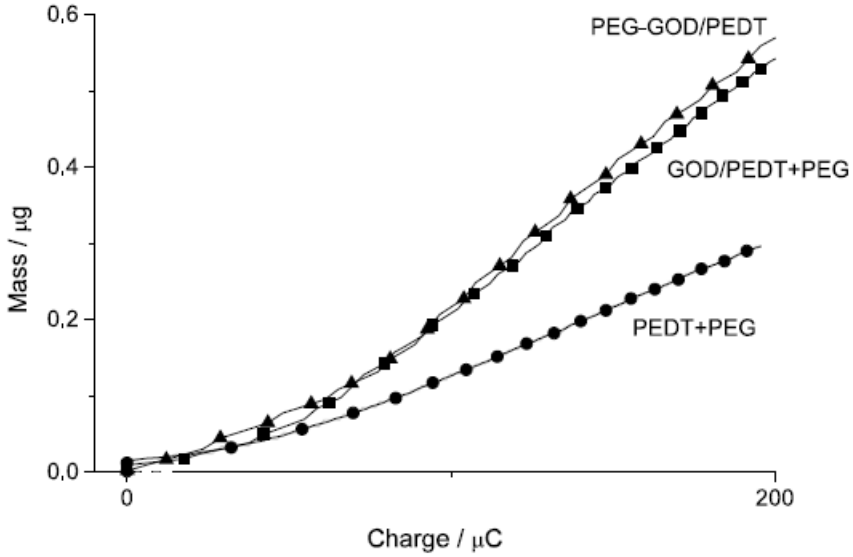


Figure 4: Data from ECQM: electrode mass versus charge during the potentiostatic electrosynthesis of (a) (PEDT+PEG) film (•), (b) (GOD/PEDT+PEG) film (•) and (c) (PEG–GOD/PEDT) film (▲). Quartz surface: 0.2 cm². Films were grown until a charge of 200 µC was reached, in 0.05 M phosphate buffer (pH 6) containing 0.05 M KCl+10⁻² M EDT+10⁻⁴ M PEG (a), 10⁻⁴ M PEG+1000 IU ml⁻¹ GOD (b) or 10⁻⁴ M PEG+1000 IU ml⁻¹ PEG–GOD (c).

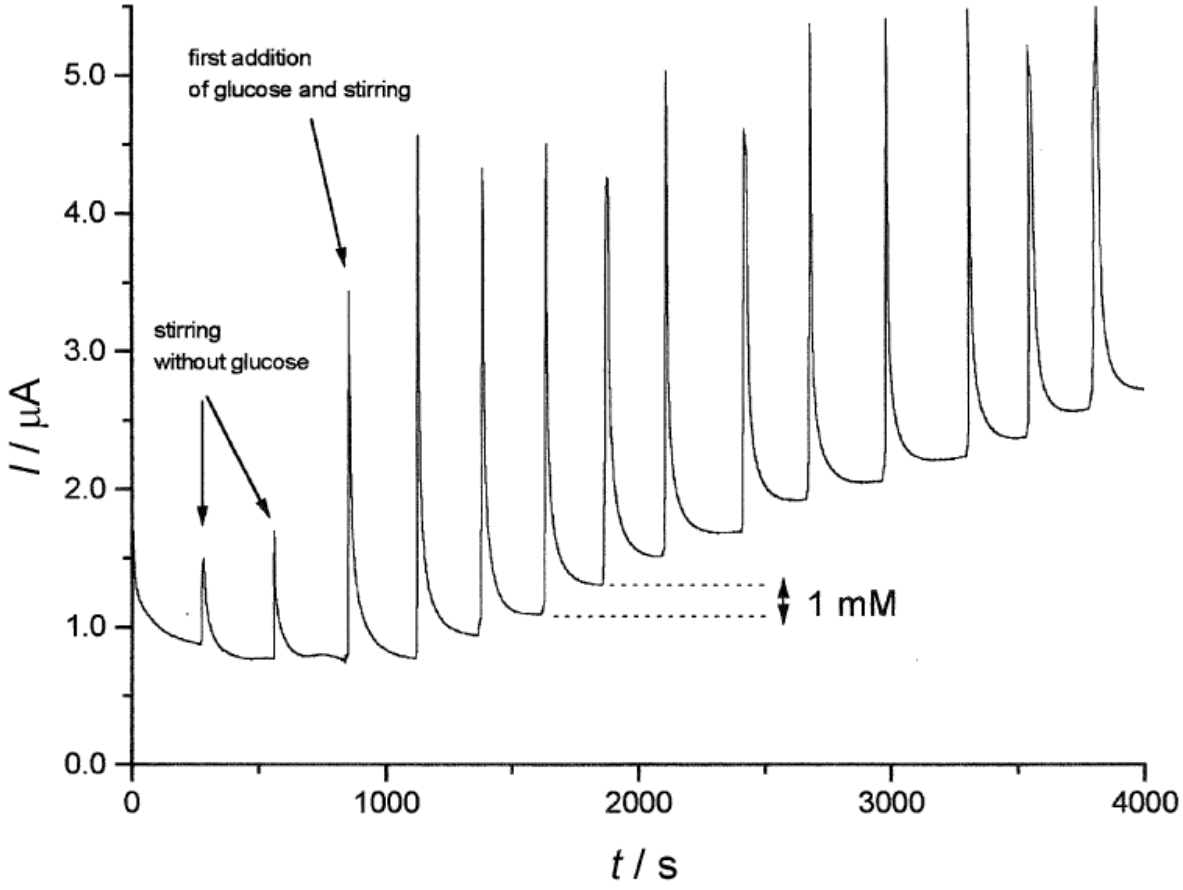


Figure 5: Amperometric response of the (PEG–GOD/PEDT) electrode versus glucose concentration (between 0 and 10 mM). E=0.3 V vs. Ag / AgCl. The medium is deaerated 0.05 M phosphate buffer (pH 6) containing 0.05 M KCl+3×10⁻⁴ M ferrocenemethanol. Successive additions (corresponding to 1 mM glucose) were injected at room temperature, under stirring for 5 s. Film thickness: 20 nm.

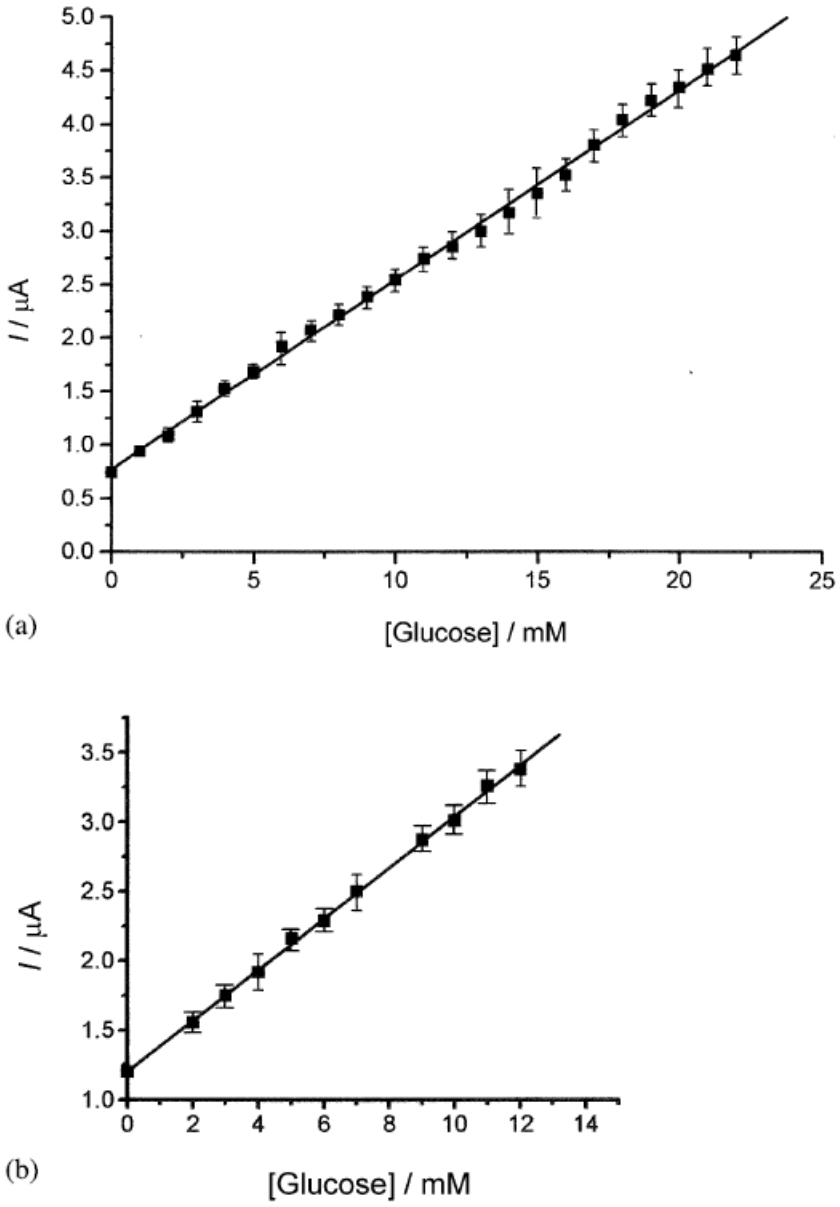


Figure 6: Calibration curves for the amperometric response versus glucose concentration for: (a) a (PEG–GOD/PEDT) film under the same conditions as Figure 5, between 0 and 22 mM, and (b) a (PEG–GOD/PEDT) electrode under aerated conditions. Each point is an average of results from three different electrodes.

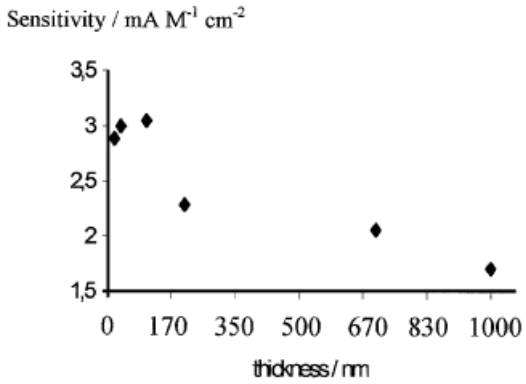


Figure 7: Sensitivity of a (PEG–GOD/PEDT) electrode under the same conditions as Figure 5, versus film thickness.

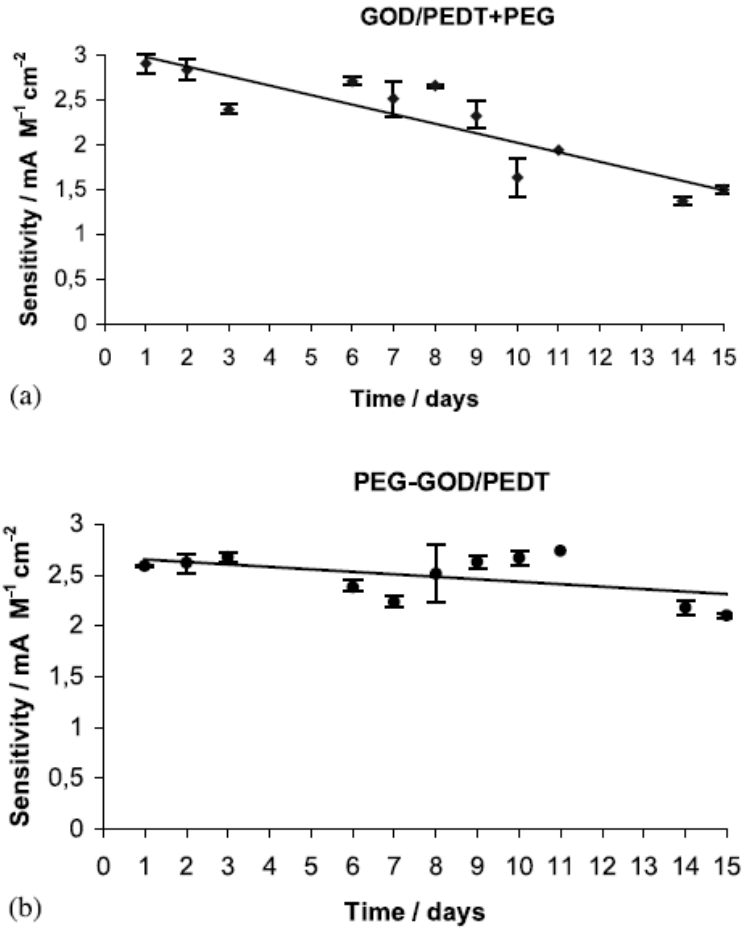


Figure 8: Sensitivities versus time (same conditions as Figure 5) for: (a) a (PEG+GOD/PEDT) electrode and (b) a (PEG–GOD/PEDT) electrode. Eleven analyses were performed during a period of 2 weeks. Each point is an average of results from three different electrodes.